

FIGURE 1a

(SEQ ID NO:1-nucleotide sequence and SEQ ID NO:2-amino acid sequence)

Escherichia coli B Phytase Sequence

1

ATG AAA GCG ATC TTA ATC CCA TTT TTA TCT CTT CTG ATT CCG TTA ACC CCG
Met Lys Ala Ile Leu Ile Pro Phe Leu Ser Leu Leu Ile Pro Leu Thr Pro

CAA TCT GCA TTC GCT CAG AGT GAG CCG GAG CTG AAG CTG GAA AGT GTG GTG
Gln Ser Ala Phe Ala Gln Ser Glu Pro Glu Leu Lys Leu Glu Ser Val Val

ATT GTC AGT CGT CAT GGT GTG CGT GCT CCA ACC AAG GCC ACG CAA CTG ATG
Ile Val Ser Arg His Gly Val Arg Ala Pro Thr Lys Ala Thr Gln Leu Met

CAG GAT GTC ACC CCA GAC GCA TGG CCA ACC TGG CCG GTA AAA CTG GGT TGG
Gln Asp Val Thr Pro Asp Ala Trp Pro Thr Trp Pro Val Lys Leu Gly Trp

CTG ACA CCG CGN GGT GGT GAG CTA ATC GCC TAT CTC GGA CAT TAC CAA CGC
Leu Thr Pro Arg Gly Gly Glu Leu Ile Ala Tyr Leu Gly His Tyr Gln Arg

CAG CGT CTG GTA GCC GAC GGA TTG CTG GCG AAA AAG GGC TGC CCG CAG TCT
Gln Arg Leu Val Ala Asp Gly Leu Leu Ala Lys Lys Gly Cys Pro Gln Ser

GGT CAG GTC GCG ATT ATT GCT GAT GTC GAC GAG CGT ACC CGT AAA ACA GGC
Gly Gln Val Ala Ile Ile Ala Asp Val Asp Glu Arg Thr Arg Lys Thr Gly

GAA GCC TTC GCC GCC GGG CTG GCA CCT GAC TGT GCA ATA ACC GTA CAT ACC
Glu Ala Phe Ala Ala Gly Leu Ala Pro Asp Cys Ala Ile Thr Val His Thr

CAG GCA GAT ACG TCC AGT CCC GAT CCG TTA TTT AAT CCT CTA AAA ACT GGC
Gln Ala Asp Thr Ser Ser Pro Asp Pro Leu Phe Asn Pro Leu Lys Thr Gly

GTT TGC CAA CTG GAT AAC GCG AAC GTG ACT GAC GCG ATC CTC AGC AGG GCA
Val Cys Gln Leu Asp Asn Ala Asn Val Thr Asp Ala Ile Leu Ser Arg Ala

GGA GGG TCA ATT GCT GAC TTT ACC GGG CAT CGG CAA ACG GCG TTT CGC GAA
Gly Gly Ser Ile Ala Asp Phe Thr Gly His Arg Gln Thr Ala Phe Arg Glu

CTG GAA CGG GTG CTT AAT TTT CCG CAA TCA AAC TTG TGC CTT AAA CGT GAG
Leu Glu Arg Val Leu Asn Phe Pro Gln Ser Asn Leu Cys Leu Lys Arg Glu

AAA CAG GAC GAA AGC TGT TCA TTA ACG CAG GCA TTA CCA TCG GAA CTC AAG
Lys Gln Asp Glu Ser Cys Ser Leu Thr Gln Ala Leu Pro Ser Glu Leu Lys

GTG AGC GCC GAC AAT GTC TCA TTA ACC GGT GCG GTA AGC CTC GCA TCA ATG
Val Ser Ala Asp Asn Val Ser Leu Thr Gly Ala Val Ser Leu Ala Ser Met

CTG ACG GAG ATA TTT CTC CTG CAA CAA GCA CAG GGA ATG CCG GAG CCG GGG
Leu Thr Glu Ile Phe Leu Leu Gln Gln Ala Gln Gly Met Pro Glu Pro Gly

TGG GGA AGG ATC ACC GAT TCA CAC CAG TGG AAC ACC TTG CTA AGT TTG CAT
Trp Gly Arg Ile Thr Asp Ser His Gln Trp Asn Thr Leu Leu Ser Leu His

AAC GCG CAA TTT TAT TTG CTA CAA CGC ACG CCA GAG GTT GCC CGC AGC CGC
Asn Ala Gln Phe Tyr Leu Leu Gln Arg Thr Pro Glu Val Ala Arg Ser Arg

GCC ACC CCG TTA TTG GAT TTG ATC ATG GCA GCG TTG ACG CCC CAT CCA CCG
Ala Thr Pro Leu Leu Asp Leu Ile Met Ala Ala Leu Thr Pro His Pro Pro

FIGURE 2
pH/Temperature Profile and Stability

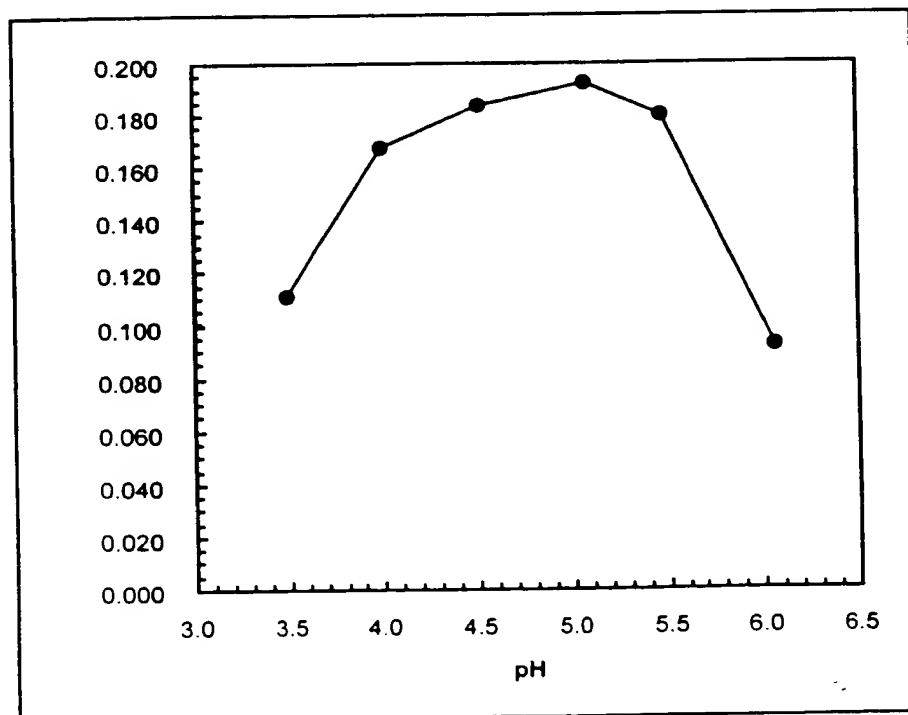
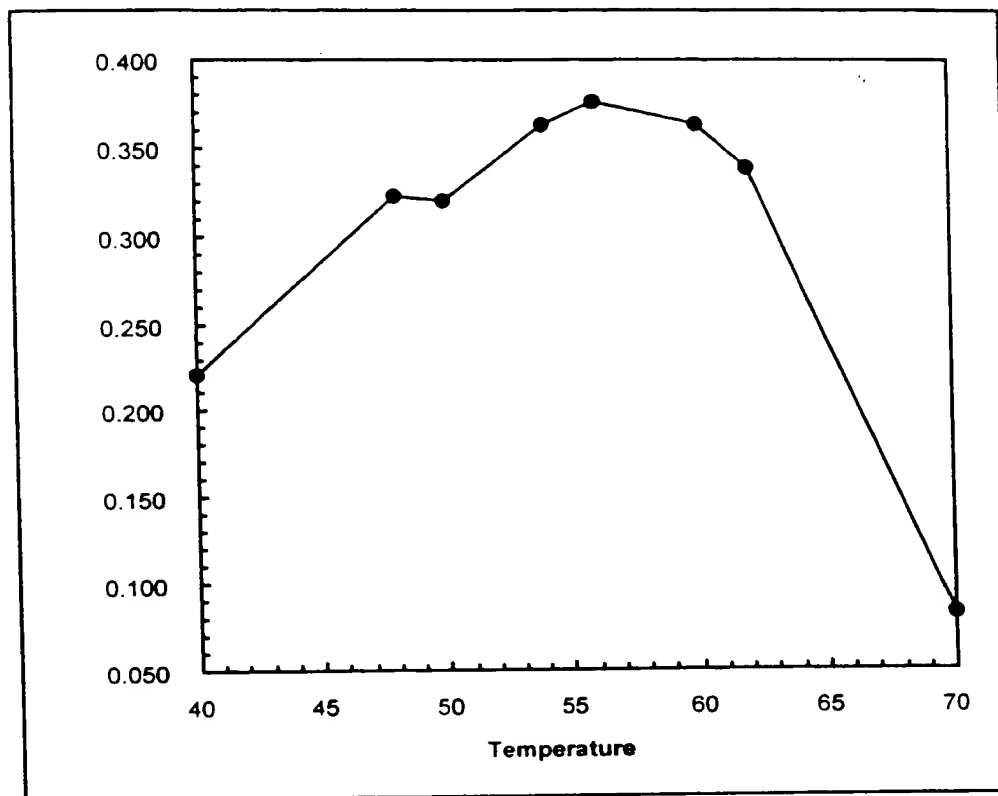


Figure 3:

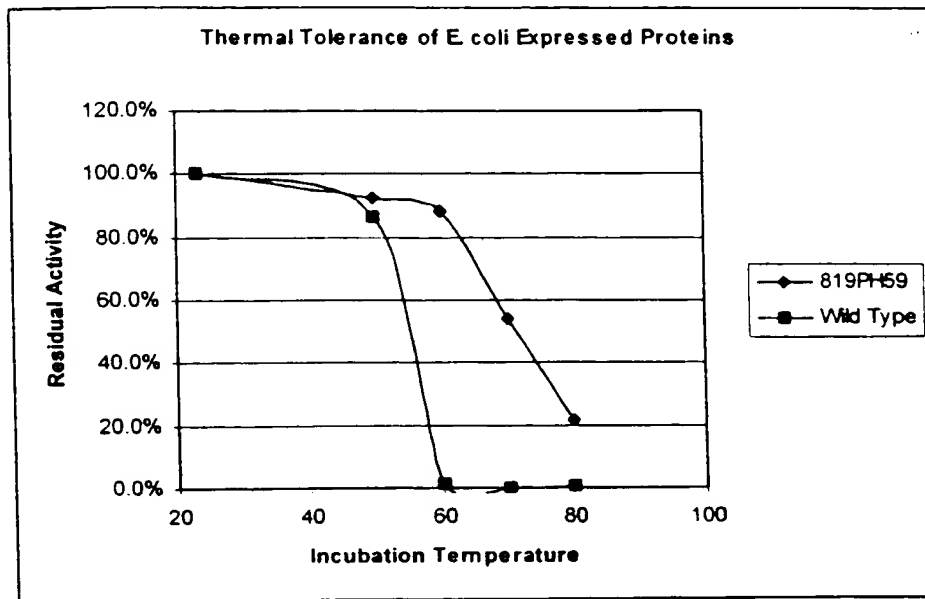


Figure 4

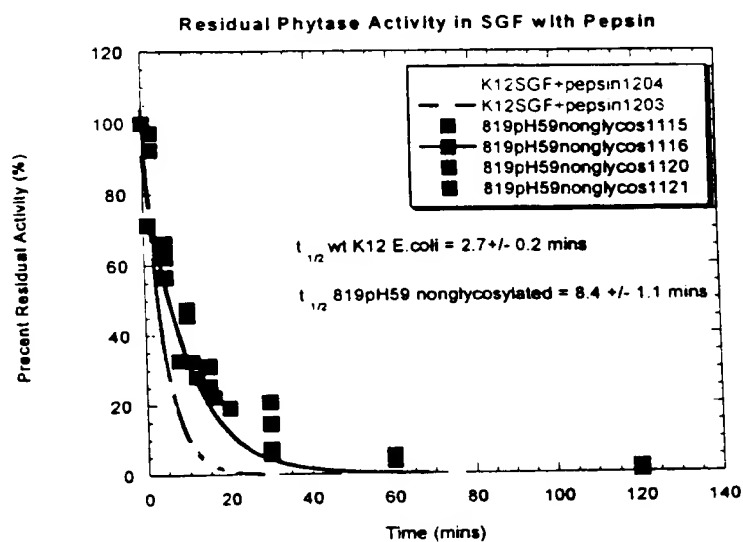


Figure 5:

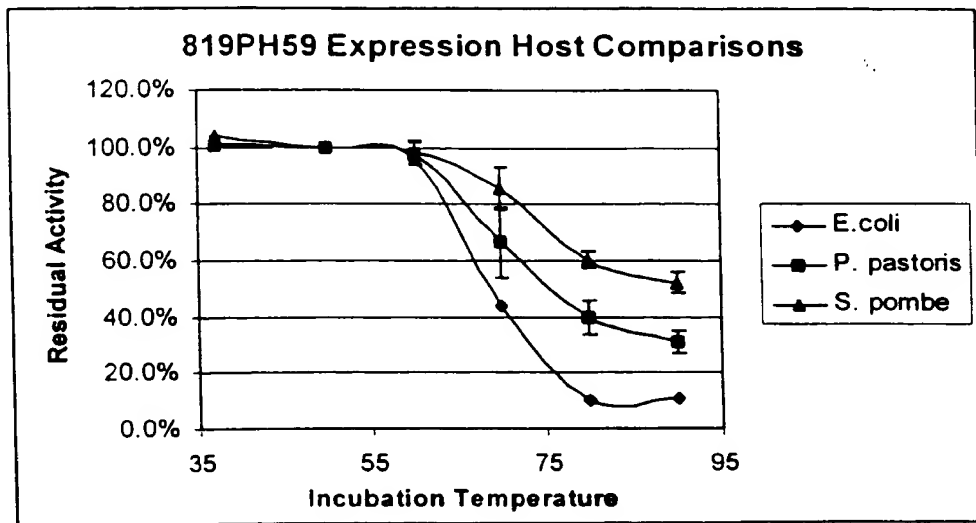


Figure 6.

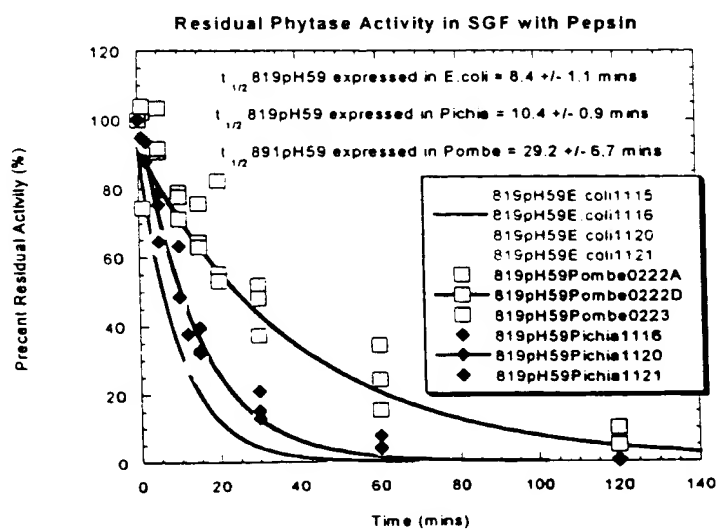


Figure 7a

E. coli appA (GenBank accession no. M58708) (SEQ ID NO:7)

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121 tgatgcggcg cattagcatc gcatcaggca atcaataatg tcagatatga aaagcggaaa
181 catatcgatg aaagcgatct taatcccatt ttatctctt ctgattccgt taaccccga
241 atctgcattc gctcagagtg agccggagct gaagctggaa agtgtggtga ttgtcagtcg
301 tcatgggtgtg cgtgctcaa ccaaggccac gcaactgatg caggatgtca cccagacgc
361 atggccaacc tggccggtaa aactgggttg gctgacaccg cngngtggg agctaatacg
421 ctatctcgga cattaccaac gccagcgtct ggtagccgac ggattgctgg cgaaaaagg
481 ctgcccgcag tctggtcagg tcgcgattat tgcgatgtc gacgagcgt cccgtaaaac
541 aggcgaagcc ttcgccgccc ggctggcacc tgaactgtca ataaccgtac ataccaggg
601 agatacgtcc agtcccgatc cgttatttaa tctctaaaa actggcggtt gccaaactga
661 taacgcgaac gtgactgacg cgatcctcag cagggcagga gggtaattg ctgactttac
721 cgggcatcgg caaacggcgt ttcgcgaact ggaacgggtg cttaatctc cgcaatcaa
781 ctgtgcctt aaacgtgaga aacaggacga aagctgttca ttaacgcagg cattaccatc
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901 gctgacggag atatttctcc tgcaacaagc acagggaatg ccggagccgg ggtggggaag
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1081 gacagcgttg acgccccatc caccgcaaaa acaggcgat ggtgtgacat taccacttc
1141 agtgcgtgtt atcgccggac acgatactaa tctggcaaat ctggcgggcg cactggagct
1201 caactggacg ctccccgtc agccggataa cagcccgcca ggtggtgaac tgggtttga
1261 acgctggcgt cggctaagcg ataacagcca gtggattcag gtttcgtgg tcttcagac
1321 ttacagcag atgcgtgata aaacgccgct gtcattaaat acgcccccc gagaggtgaa
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1441 tacgcaaata gtgaatgaag cagcctatcc ggctgcagt ttgtaatga taaaaagag
1501 cattcagta cctgaatgct ctgaggctga tgacaaacga agaactgtct aatgcgtaga
1561 ccggaaaagg cgttcacgcc gcatccggcc acttcagtt ttctcttc tcggagtaac
1621 tataaccgta atagttatag ccgtaactgt aagcgggtgt ggccggtta atcacacat
1681 tgaggatagc gcctttaata ttgacgcctg cctgttcag acgtgcatt gacaaactca
1741 cctcttggc ggtgttcaag ccaaacgcg caaccagcag gctggtgcca acagaacgcc
1801 ccacgaccgc ggcatcactc accgccagca tcggcggcgt atcgacaatc accagatcgt
1861 aatggtcgtt cgccattcc agtaattgac gcatccgatc g
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Figure 7b

1 taaggagcag aaacaatgtg gtatttactt tggttcgtcg gcattttgtt gatgtgttcg
61 ctctccaccc ttgtgttggg atggctggac ccgcgtctga aaagttaacg aacgtaggcc
121 tgatgcggcg cattagcatc gcatcaggca atcaataatg tcagatatga aaagcggaaa
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481 ctgcccgcag tctggtcagg tcgcgattat tctgatgtc gacgagcgta cccgtaaaa
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721 cgggcatcgg caaacggcgt ttgcgaact ggaacgggtg cttaatttc cgcaatcaaa
781 ctgtgctt aaacgtgaga aacaggacga aagctgtca ttaacgcagg cattaccatc
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901 gctgacggag atattctcc tgaacaagc acagggaatg ccggagccgg ggtggggaag
961 gatcaccgat tcacaccagt ggaacacctt gctaagttg cataacgcgc aattttatt
1021 gctacaagc acgccagagg ttgcccgcag ccgcgccacc ccgttattag attgatcaa
1081 gacagcgtt acgccccatc caccgcaaaa acaggcgtat ggtgtgacat taccacttc
1141 agtgcgtgtt atcgccggac acgatactaa tctggcaaat ctggcgccg cactggagct
1201 caactggacg ctcccggtc agccggataa cacgccgcca ggtggtgaac tgggtttga
1261 acgctggcgt cggctaagcg ataacagcca gtggattcag gttcgttg tcttcagac
1321 ttacagcag atgcgtgata aaacgccgt gtcattaaat acgccgccg gagaggtgaa
1381 actgacctg gcaggatgtg aagagcgaaa tgcgcagggc atgtgtcgt tggcagggtt
1441 tacgcaaate gtgaatgaag cagcatacc ggcgtgcagt ttgtaatgca taaaaaagag
1501 cattcagta cctgaatgct ctgaggctga tgacaaacga agaactgtct aatgcgtaga
1561 ccggaagg cggtcacgcc gcatccggcc acttcagtt ttctcttc tggagtaac
1621 tataaccgta atagttatag ccgtaactgt aagcgggtgct ggcgcgttta atcacaccat
1681 tgaggatagc gccttaata ttgacgcctg cctgttcag acgctgcatt gacaaactca
1741 cctctttggc ggtgttcaag ccaaaacgcg caaccagcag gctggtgcca acagaacgcc
1801 ccacgaccgc ggcatcactc accgccagca tcggcgccgt atcgacaatc accagatcgt
1861 aatggtcgtt cgccattcc agtaattgac gcatccgac g

Figure 8

Amino acid sequence for *E. coli* appA (wild type) (SEQ ID NO:8)

MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVT
PDAWPTWPVKLGWLTPRGGELIAYLGHYQRQRLVADGLLAKKGCPQSGQVA
IADVDERTRKTEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDNA
NVTDAILSRAGGSIADFTGHRRQTAFRELERVLNFPQSNLCLKREKQDESCSLTQ
ALPSELKVSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNT
LLSLHNAQFYLLQRTPEVARSRATPLLDLIKTAALTPHPPQKQAYGVTLPSTVLF
AGHDTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSL
VFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEARI
PACSL

Bold-Underlined amino acid residues are shown below in the modified appA enzyme
(SEQ ID NO:10)

MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVT
PDAWPTWPVKLGELTPRGGELIAYLGHYWRQRLVADGLLPKCGCPQSGQVAI
IADVDERTRKTEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDNA
NVTDAILERAGGSIADFTGHYYQTAFRELERVLNFPQSNLCLKREKQDESCSLTQ
ALPSELKVSADCVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNT
LLSLHNAQFDLLQRTPEVARSRATPLLDLIKTAALTPHPPQKQAYGVTLPSTVLF
AGHDTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSL
VFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEARI
PACSL